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Concomitant Chronic Lymphocytic Leukemia and Acute Myeloid Leukemia With an Uncommon Immunophenotype

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We report a case of simultaneous diagnosis of chronic lymphocytic leukemia (CLL) and acute myeloid leukemia (AML), in which the use of flow cytometry analysis allowed the demonstration of two different cell populations and the study of both immunophenotyping patterns with a large panel of monoclonal antibodies (MoAbs). CLL cells showed a typical immunophenotype with coexpression of B cell markers with CD5, CD23, CD43, and weak surface immunoglobulin light chain restriction expression, whereas the AML population had a very uncommon phenotype with expression of myeloid markers and CD56 and lack of expression of other natural killer (NK) antigens, CD34 and HLA-DR. After chemotherapeutic treatment of AML with two induction courses, the patient achieved complete remission of the AML with persistence of a CD19/CD5 positive population. After consolidation chemotherapy, this latter population was no longer detectable despite the presence of lymphoid nodules in a bone marrow biopsy. Six months after diagnosis, the patient relapsed with AML and died shortly afterwards. Am. J. Hematol. 56:281–287, 1997. © 1997 Wiley-Liss, Inc.

Key words: acute myeloid leukemia; chronic lymphocytic leukemia; immunophenotyping

INTRODUCTION

The association of chronic lymphocytic leukemia (CLL) and acute leukemia (AL), either lymphoid or myeloid, is a rare event [1]. It has been suggested that AL may occur as a secondary leukemia following treatment with chemotherapy and/or radiotherapy [1,2] or in some cases of lymphoblastic leukemia, sharing the same monoclonal surface immunoglobulin, as part of the natural history of CLL [3,4]. In some patients, acute myeloid leukemia (AML) has been diagnosed simultaneously with CLL [5–15] or during the course of an untreated case of CLL [5,16–19]. Most of these cases have been diagnosed on the basis of morphology and cytochemistry and little information is available about the immunophenotypic profiles of these two cell populations.

We report a case of concomitant detection of CLL and AML in which a detailed immunophenotypic study of both cell populations was performed by flow cytometric analysis.

CASE REPORT

A 59-year-old man was referred for evaluation of spontaneous cutaneous bleeding, hematuria, and fever. Past medical and surgical history was remarkable for non-insulin dependent diabetes mellitus, hypertension, and appendicectomy. He had no history of exposure to ionizing radiation or toxic chemicals. On physical examination the patient appeared febrile and hypertensive, with purpura in the extremities. There was no lymphadenopathy, splenomegaly, or hepatomegaly. Laboratory studies revealed a hematocrit of 38%, platelet count of 31×10^9/l, and a white cell count of 246×10^9/l (2% neutrophils, 5% lymphocytes, 93% blasts), creatinine 120 μmol/l, lactate dehydrogenase 1414 U/l, and uric acid 504 μl/l. Chest radiogram was normal. A diagnostic bone marrow aspirate was performed and a portion of the aspirate material was submitted for flow cytometric and cytogenetic analysis. Results of light microscopic examination and flow cytometric analysis were diagnostic for both AML-M1, according to the FAB classification [20],

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and B-cell CLL. A chemotherapeutic regimen that included idarubicin 10 mg/m^2 iv days 1, 3, and 5, etoposide 100 mg/m^2 iv days 1–3, and cytarabine 25 mg/m^2 and 100 mg/m^2 iv in continuous infusion days 1–7 was begun. Complications included fever empirically treated with antibiotics and antifungal agents and grade II renal insufficiency according to the WHO grading system. A bone marrow aspirate performed on day 28 showed persistence of both AML and CLL. At that time, the peripheral blood count showed a hematocrit of 27%, platelets 131 × 10^9/l, and leukocytes 9.22 × 10^9/l (4% bands, 26% neutrophils, 1% basophils, 29% lymphocytes, 40% blasts). The patient was considered refractory and a course of salvage chemotherapy including mitoxantrone 12 mg/m^2 iv days 1–3, cytarabine 500 mg/m^2 iv as a 24-hr continuous infusion days 1–3 and 6–8, etoposide 100 mg/m^2/12 hr iv days 6–8, and granulocyte-colony stimulating factor (G-CSF) 50 μg/m^2/d sc was started. Post-chemotherapy complications included: Grade IV mucositis, grade III gastrointestinal toxicity, grade II renal insufficiency, persistent fever with a positive blood culture for ''Enterococcus faecium'' and skin toxicity due to vancomycin. A new bone marrow study performed on day 28 showed complete remission (CR) of AML with persistence of CLL. The peripheral blood count at the time showed no lymphocytosis. A consolidation phase was administered with etoposide 100 mg/m^2 12 hr iv days 1–3, cytarabine 2 g/m^2/12 hr iv days 1–5, and G-CSF 5 μg/kg/d sc from day 6 to hematologic recovery. The patient presented reversible cerebelar toxicity, probably secondary to cytarabine, and neutropenic fever with enterocolitis, which was treated with antibiotics and antifungal agents. A bone marrow biopsy after consolidation showed persistence of AML remission and several lymphoid nodules. Six months after diagnosis, the patient relapsed with AML and died shortly afterwards. Autopsy was not performed.

MATERIALS AND METHODS

Light Microscopic Preparations

The peripheral blood and bone marrow aspirate smears were stained with May-Grünwald-Giemsa. Aspirate smears were also stained for the presence of myeloperoxidase (MPO), chloroacetate esterase, and α-naphtyl acetate esterase. The bone marrow biopsy was fixed and decalcified in 4% formic acid, embedded in paraffin, and stained with hematoxylin-eosin and Giemsa.

Flow Cytometric Immunophenotyping

Sample preparation. One hundred microliters of heparinized bone marrow material or peripheral blood with 1 × 10^6 cells were aliquoted into each staining tube. Aliquots for surface immunoglobulin staining were washed twice with 4 ml of phosphate buffered saline (PBS) and incubated at 37°C for 1 hr. Appropriate volumes of fluorescein isothiocyanate (FITC) or phycoerytrin (PE) conjugated monoclonal antibodies (MoAbs) were added to each tube. The tubes were incubated at room temperature in the dark for 15 min. Two milliliters of lysing solution [FACSLyse, Becton Dickinson Immunocytometry Systems (BDIS), San Jose, CA] were added to each tube. The samples were vortexed and allowed to incubate at room temperature for 10 min, and then centrifuged (200*g*) to obtain a pellet. Each aliquot was washed once with 2 ml PBS and resuspended in 0.5 ml of PBS prior to flow cytometric analysis. For intracellular antigen detection, we used a Fix & Perm cell permeabilization kit (Caltag, San Francisco, CA).

Monoclonal antibodies. The panel used included CD2 (Becton Dickinson [BD]) (BD), CD3 (BD), CD4 (BD), CD5 (BD), CD7 (BD), CD8 (BD), CD10 (BD), CD11b (BD), CD13 (BD), CD14 (BD), CD16 (BD), CD19 (BD), CD20 (BD), CD21 (Serotec Ltd, Oxford, England) (ST), CD23 (Immunotech, Marseille, France) (I), CD33 (BD), CD34 (I), CD36 (I), CD41 (ST), CD43 (ST), CD56 (BD), CD57 (BD), CD61 (BD), FMC7 (Sera-Lab Ltd, Sussex, England) (SL), HLA-DR (BD), Glycophorin A (I), cytoplasmic (C) MPO (Dakopatts, Glostrup, Denmark), cCD3 (BD), nuclearTdT (SL), immunoglobulin light chains (BD). Controls were provided by cells incubated with irrelevant FITC and PE mouse immunoglobulins.

Flow cytometry. Samples were analysed on a FACScan flow cytometer (BD) using a LYSIS II and PAINT-A-GATE software programs. Forward (FSC) and sideward (SSC) light scatter were collected using linear amplification. Ten thousand events were acquired using logarithmic amplification for green and red fluorescence. Cells were analyzed setting gates on the lymphocyte and blast populations. The quadrant markers designating positivity for FITC and PE were drawn according to the position of the negative population on the isotype control plot. A case was defined as positive when >20% of cells were positive for the MoAb.

Cytogenetic Studies

Cytogenetic study was performed on bone marrow cells after short-term culture without stimulation, at diagnosis and on day 28 after the first induction treatment. Chromosomes were banded using G-banding and chromosomal abnormalities were described according to the ISCN nomenclature [21].

RESULTS

Morphologic Features

The initial bone marrow aspirate showed a hypercellular bone marrow with 80% blasts and 18% lymphocytes. The blasts were medium-large with a high nuclear/

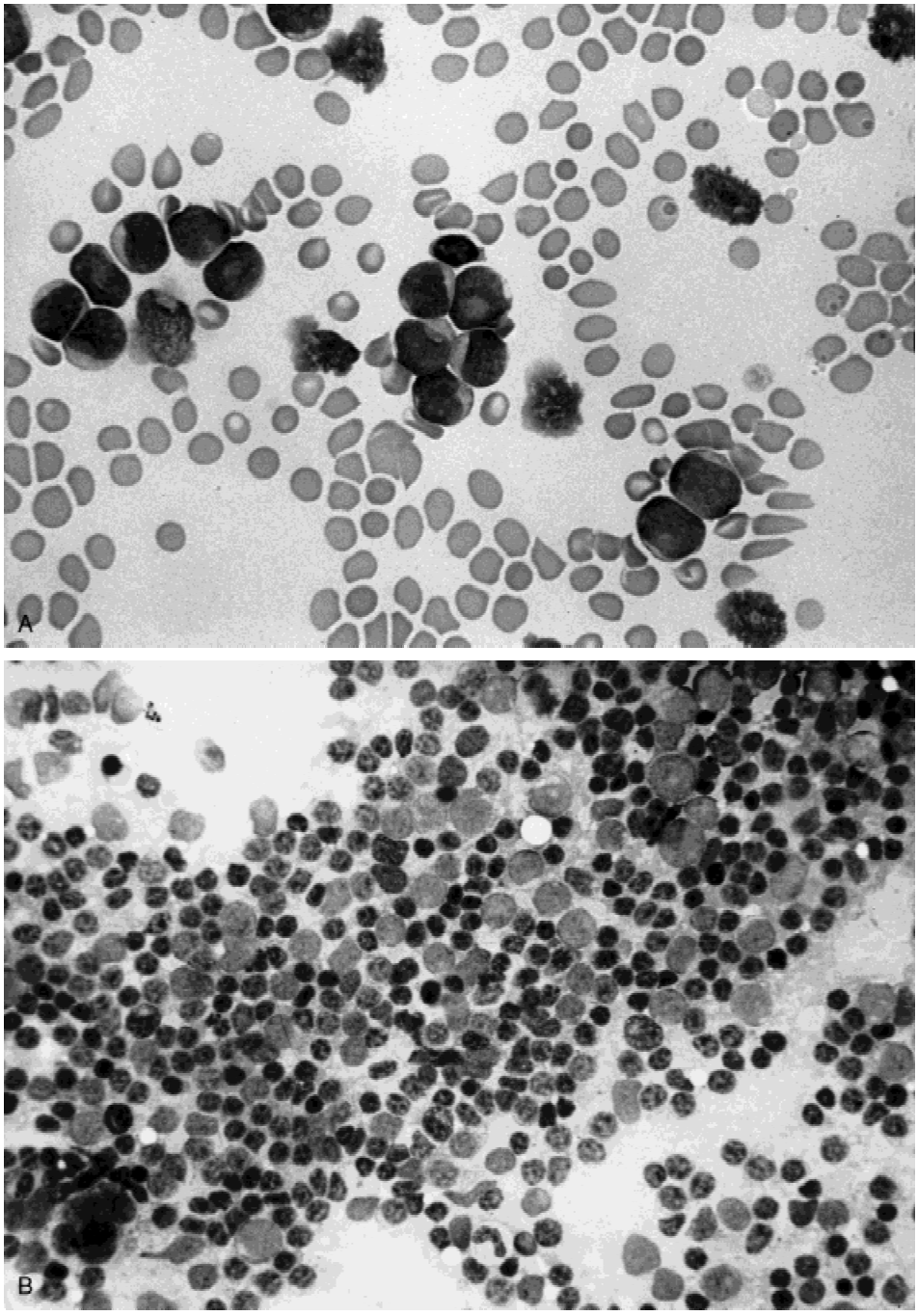

Fig. 1. Peripheral blood smear (A) (May-Grünwald Giemsa, ×400) and bone marrow smear (B) (May-Grünwald Giemsa, ×200) showing two different populations of cells: chronic lymphocytic leukemia-appearing lymphocytes and blast cells.

cytoplasmic ratio. Nuclei had an open chromatin with inconspicuous nucleoli. Few cytoplasmic granules were present in some blasts (Fig. 1A). MPO was positive in 80% of the blast population whereas chloroacetate esterase and α-naphtyl acetate esterase were negative. Lymphocytes were small, with round nuclei and clumped chromatin (Fig. 1A). The second bone marrow aspirate showed a hypercellular bone marrow with blasts and CLL-appearing lymphocytes (Fig. 1B). In the third study no blasts were observed but 17% of lymphocytes with lymphoid aggregates were present. The bone marrow biopsy after consolidation showed persistence of AML remission with lymphoid nodules (Fig. 2).

Immunophenotype

At diagnosis, flow cytometry analysis demonstrated two different populations according to the FSC and SSC

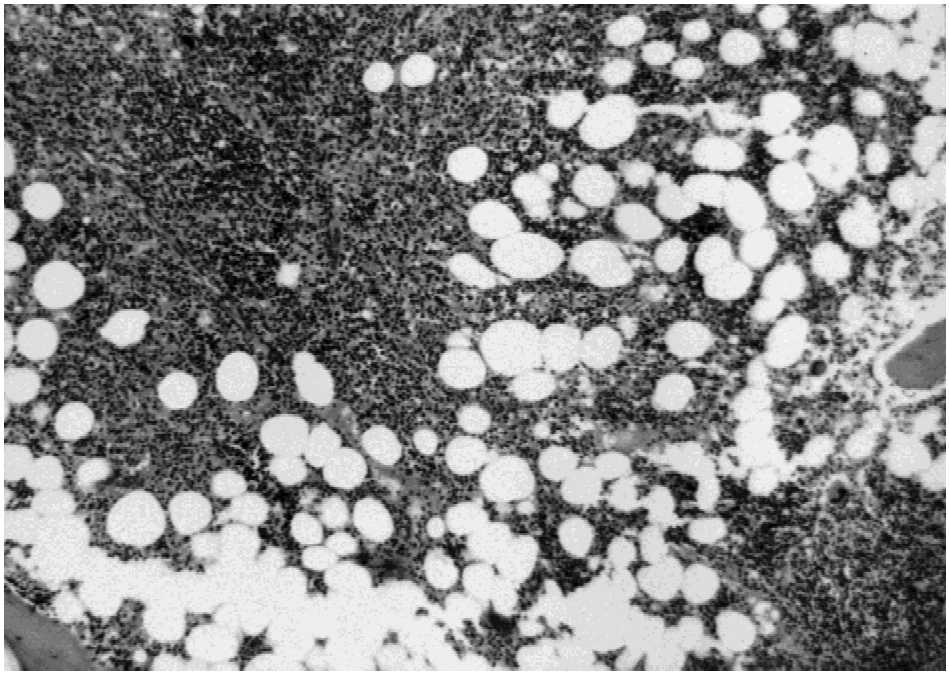

Fig. 2. Lymphoid aggregates in the bone marrow biopsy performed after consolidation (hematoxylin-eosin, ×100).

scatter characteristics with distinct immunophenotypic features that corresponded to the lymphocytes and the blast population (Fig. 3). Bone marrow lymphocytes were positive for B-cell markers (CD19, CD20, CD21), CD5, CD23, and CD43. The blast population was positive for CD4 (faint intensity), CD13, CD33, CD56, and cMPO (Table I). Peripheral blood lymphocytes disclosed a population of residual mature T lymphocytes (60%) (CD8 40%, CD4 17%) and a population of B lymphocytes (30%) that coexpressed CD5, CD23, CD43, and kappa light chains (faint intensity) as demonstrated by dual-staining experiments. When AML remission was obtained (third aspirate), immunophenotypic studies showed persistence of a CD5/CD19 positive lymphocyte population. This population could not be demonstrated in the bone marrow aspirate carried out at the end of treatment, although lymphoid nodules were observed in the biopsy specimen.

Cytogenetic Analysis

No metaphases were available at diagnosis. Four of 60 metaphases from the second bone marrow sample showed nonclonal chromosomal abnormalities. The karyotype was: 46, XY, del(7)(q22). 46,XY, t(1;4)(p36;q26). 46, XY, t(6;7)(p23;p15). 46, XY, t(1;9)(q25;p23), respectively.

DISCUSSION

We report a patient who presented with concomitant CLL and AML-M1. The two different populations were identified on the basis of cell morphology and immunophenotyping.

Most cases of AML diagnosed concomitantly or in the course of an untreated CLL were published prior to the development of MoAbs. Clonality of the lymphocyte population defined as restriction in the pattern of expression of light chains has therefore seldom been demonstrated [8,11,14,15,17] and immunophenotype of the blast population reported in few cases [13–15,18]. The large panel of MoAbs studied in our case defined the typical B-cell CLL phenotype (B cell markers with coexpression of CD5, CD23, and CD43) and the following phenotype in the blast population: CD33+, CD13+, CD56+, cMPO+, CD34−, HLA-DR−, CD16−, CD57−. Coexpression of myeloid markers with CD56, a natural killer (NK) cell-associated antigen, with lack of expression of other NK cell antigens and HLA-DR has only been described in 6% of adult de novo AML [22]. The 20 patients reported by Scott et al. [22] included 10 cases initially classified as AML-M1 although in the morphological review performed by the authors, 13 cases were described as having morphologic features similar to those of the microgranular variant of acute promyelocytic leukemia. These blast characteristics, with deeply invaginated nuclear membranes and scant cytoplasm with fine azurophilic granularity, were not observed in our patient. Interestingly, 4/18 cases of the series reported by Scott et al. [22] had lymphocyte counts above 4×10^9/l, although no immunophenotypic data for this lymphocyte population were available. Scott et al. [22] described these cases as myeloid/NK cell acute leukemias on the basis of the positivity for myeloid markers

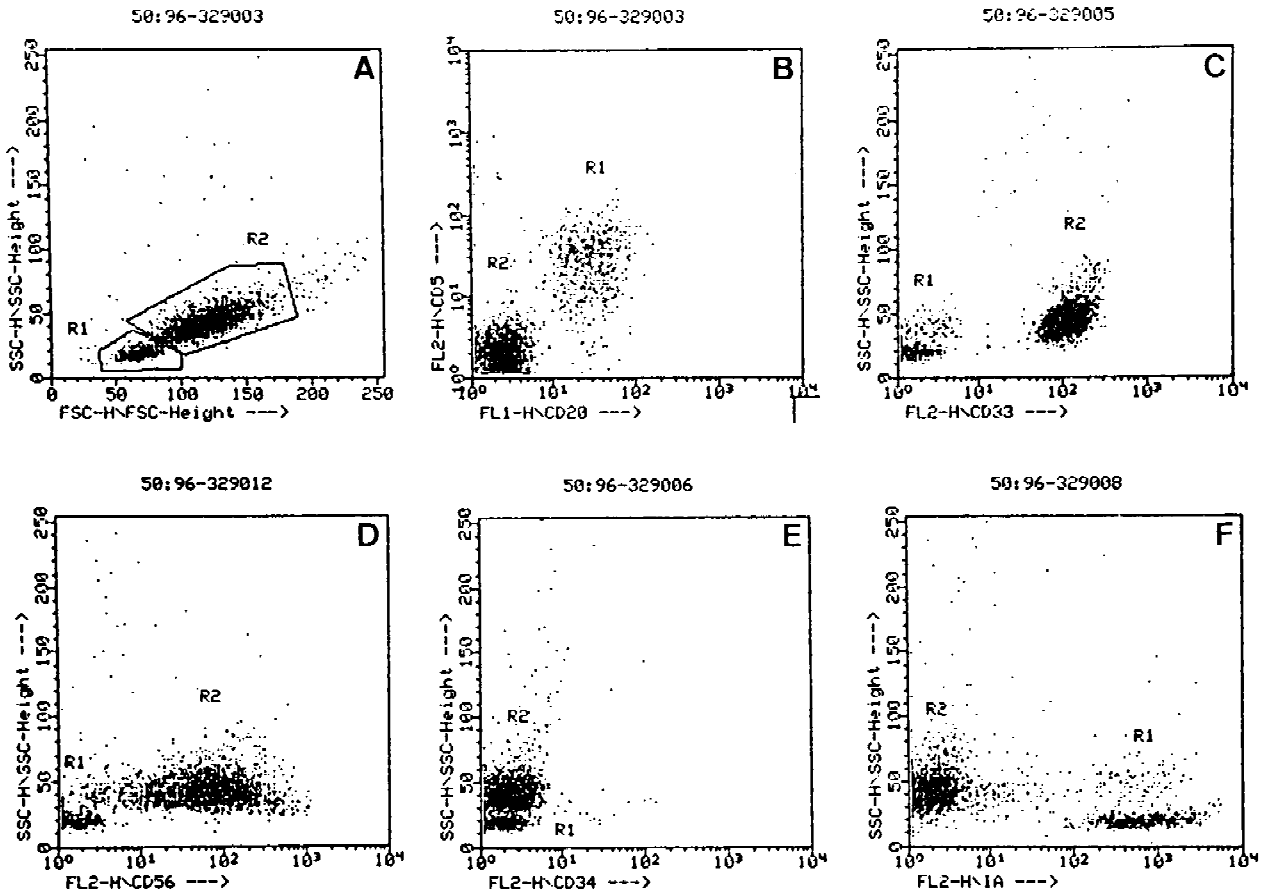

Fig. 3. A: Forward scatter (FSC) vs. side scatter (SSC) dot plot showing two different populations, lymphocytes (R1) and blasts (R2). B: Lymphocytes (R1) coexpressed CD5 and CD20, as determined by dual-staining analysis. Blasts (R2) expressed CD33 and CD56 whereas they didn't express CD34 and HLA-DR (IA). Lymphocytes (R1) didn't express CD33, CD56 and CD34 but express HLA-DR (IA), as determined using the PAINT-A-GATE program (C–F).

and CD56 and demonstration of a functional NK cell-mediated cytotoxicity in four of six cases tested. These authors suggest this form of AL may arise from transformation of a precursor cell common to both the myeloid and NK cell lineages, and demonstrate a normal CD56+, CD33+, CD16− counterpart cell at a frequency of 1–2% in the peripheral blood of healthy individuals. Although this is an attractive hypothesis, it should be stated that CD56 has been reported to be positive in 22% of all AML cases (50% of these cases were also CD34 negative) and in 19% of AML-M1 cases [23]. Other authors have reported 36% of CD56 positive cases in the phenotypic group CD13+/CD33+, HLA-DR− [24].

Cytogenetic study in our patient did not show any clonal chromosomal abnormality. Cytogenetics were normal in most of the cases reported by Scott et al. [22]. On the other hand, cytogenetic studies were described in only three previous cases of concomitant AML and CLL, reporting no recurrent anomaly [11,13,19]. Additionally, in no case has the same cytogenetic aberration been reported in both the CLL and AML populations, a finding that would support the hypothesis of a common aberrant pluripotent progenitor developing along two different pathways, as has been suggested by some authors [11,12,15,16,19]. Other hypotheses have been made in an attempt to explain the association of AML and untreated CLL. It is possible that some common stimulus or leukemogenic factor could affect two cell lines [6,13,15,16,18,19]. Reference is made to the high incidence of secondary malignancies in patients with CLL [5,10,11,13,15–19], probably related to impaired immune competence. Besides, multiple primary neoplasms could be due to a genetic susceptibility in certain individuals [6,15,16]. Another hypothesis is the mere chance coincidence of two diseases more commonly seen in the elderly [13,15,17–19].

With regard to the clinical evolution, after AML treatment no lymphocytosis was observed in peripheral blood in our patient and no lymphadenopathy, hepatomegaly, or splenomegaly could be detected by physical examination and serial ultrasound abdominal studies. Despite the presence of lymphocyte nodules in the bone marrow bi-

TABLE I. Immunophenotype

Cluster of differentiation	Lymphocytes	Blasts
CD2	–	–
CD3	–	–
CD4	–	+
CD5	+	–
CD7	–	–
CD10	–	–
CD11b	–	–
CD13	–	+
CD14	–	–
CD16	–	–
CD19	+	–
CD20	+	–
CD21	+	–
CD23	+	–
CD33	–	+
CD34	–	–
CD36	–	–
CD41	–	–
CD43	+	+
CD56	–	+
CD57	–	–
CD61	–	–
FMC7	–	–
HLA-DR	+	–
Glycophorin A	–	–
c MPO[a]	–	+
cCD3	–	–
Nuclear TdT	–	–

[a]c: cytoplasmic; MPO: myeloperoxidase.

opsy, no CD19/CD5 positive lymphocytes could be isolated in either the peripheral blood or bone marrow aspirate by flow cytometric analysis. This was also the case at the time of AML relapse. Response of CLL to chemotherapeutic treatment for AML has been assessed by immunophenotypical investigations only in one previous patient [14]. In this case, CR for CLL was achieved with a regimen including high-dose cytarabine, although it is not reported whether or not a bone marrow biopsy was performed in order to rule out the existence of lymphoid nodules, as in our case. It is interesting to point out that in the case reported by Bracey et al. [19], AML developed from a previous myelodysplastic syndrome shortly after treatment of the CLL. The authors suggested that lymphocyte down-regulation of the myeloid clone could explain this evolution. This case and others [25] raise the question of the eventual interaction between the CLL and the myeloid population and how it could be affected by treatment.

The systematic application of flow cytometric studies will demonstrate whether the incidence of this association has been previously underestimated, as it is possible that CLL lymphocytes could be misdiagnosed as reactive lymphocytes. Moreover, the application of a large panel of MoAbs could define specific phenotypes for AML cases associated with CLL that, together with systematic application of other techniques such as cytogenetics, will provide a new approach to the pathogenesis of this association.

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